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(54) Title: DNA SEQUENCES FROM SPECIFIC HUMAN GENOMIC LOCI USEFUL FOR IDENTIFICATION OF INDIVIDUALS

(57) Abstract

A novel method for determining the genetic identity between two samples is provided. The method allows sizing of the VNTR regions of the above loci for samples of DNA which can be used to establish or exclude identity between samples. The determination advantageously uses oligonucleotide probes or primers capable of hybridizing with various regions of loci selected from the group consisting of D18S17, D1S47, D20S15, D21S112, D6S22, and D11S129.

DNA SEQUENCES FROM SPECIFIC HUMAN GENOMIC LOCI USEFUL FOR IDENTIFICATION OF INDIVIDUALS

This invention relates in general to methods and products used for identity testing and, in particular, to oligonucleotide probes and primers and methods of using such probes and primers. The methods and products of this invention are particularly useful for paternity or forensic applications.

BACKGROUND OF THE INVENTION

Conventional methods for determining identity are based typically upon an individual's blood type or fingerprints. These methods, however, have well known shortcomings. More recently, investigators have turned to genetic analysis to determine identity. Identity, including familial relationship, may be determined using genetic material with a high degree of certainty and from extremely small samples of genetic material.

Regions of the human genome that are quite variable in different individuals have been described (Wyman & White, 1980, Proc. Nat'l. Acad. Sci. USA 77: 6754- 6758; Donis-Keller et al, 1987, Cell 51: 319-337). Many of these regions of

variability or "polymorphisms" are observed by cleaving human DNA with one of a variety of sequence-specific restriction endonucleases, separating the cleaved fragments by size on a gel, transferring the DNA to a membrane, and hybridizing the DNA with probe which has been labeled with a reporter group such as ^{32}P. Such a "Southern" blot is then exposed to x-ray film to reveal the positions of bands which hybridize to the labeled probe. A difference between two DNA samples in the position of a band is indicative of a polymorphism. These polymorphisms are referred to as restriction fragment length polymorphisms (RFLP).

One class of these polymorphisms behave as though various multiples of discrete pieces of DNA had been added or deleted from the polymorphic region in different individuals. These short tandem repetitive DNA sequences are known as "minisatellites" or VNTR because they have been shown to be comprised of variable numbers of tandem repeats. One of the earliest descriptions of such a VNTR locus was published by Jarman et al. (1986, EMBO Jour. 5: 1857-1863) and described the 3' flanking region of the human beta-globin locus which contains in different individuals between 70 and 450 copies of a 17 bp repeat. Other loci containing such VNTRs have been described since that time. At the present time, over 30 such VNTR loci have been described in the literature. However, in only a few cases are the DNA sequences known. With respect to these few cases, the DNA sequence of the

repeated regions of VNTR loci differs slightly from one another, and also the DNA sequence of each repeat within a single VNTR locus varies slightly (Jeffreys et al., 1985, Nature 314: 67-73; Nakamura et al., 1987, Science 235: 1616-1622). Synthetic oligonucleotide probes have been designed with consensus sequences for several VNTR loci, and under suitably low stringency hybridization conditions, they will detect multiple VNTR loci simultaneously in a Southern blot hybridization experiment (Jeffreys et al., 1985, supra). Synthetic oligonucleotides have also been designed which detect only specific VNTR loci under suitably high stringency hybridization conditions (Ali & Wallace, 1988, Nucl. Acid. Res. 16: 8487-8496).

No function has been attributed to these repeated VNTR regions of DNA in the human genome. The fact that they can be present in variable numbers with no obvious consequence to the health of the individual suggests that they may serve little or no function.

SUMMARY OF THE INVENTION

This invention provides nucleic acid or oligonucleotide probes and primers capable of hybridizing selectively to VNTR loci and useful for determining identity. The variability of a VNTR region from one individual to another can be so high the pattern of numbers of repeats at several different genetic loci revealed by probing with a panel of different VNTR probes can be unique for a

particular individual. If the panel of probes is large enough, the identity of a sample can be determined with confidence. Thus, these patterns can be used in forensic applications, in establishing paternity, and in any application in which ability to unambiguously identify an individual is important.

This invention involves the discovery that a particular group of loci, D18S17, D1S47, D20S15, D21S112, D6S22, and D11S129, have at least one region containing a variable number of tandem repeats (hereinafter VNTR region) and the subsequent DNA sequencing of these VNTR regions and regions which flank the VNTR regions. The identification of these VNTR loci along with the sequence information provides sufficient basis for preparing oligonucleotide probes and extension primers. These probes and primers are specially designed for use in assays involving the comparative study of two samples of DNA and the determination of identity or nonidentity between the source of the two samples.

The oligonucleotide probes of the invention are capable of selectively hybridizing to a VNTR region of a locus selected from the group consisting of D18S17, D1S47, D20S15, D21S112, D6S22, and D11S129. These oligonucleotide probes may be of a size and specificity so as to hybridize only to a single repeat region within the VNTR, to two or more contiguous repeat regions within the VNTR or to the entire VNTR region. The probes of the invention

also may be of a size and specificity so as to hybridize only to regions which flank the VNTR regions or to both a flanking region and a VNTR region contiguous with one another.

The invention also provides primers for initiating the synthesis of an extension product along a strand of DNA. These primers are oligonucleotides capable of hybridizing to at least a portion of the flanking region of a VNTR region of a locus selected from the group consisting of D18S17, D1S47, D20S15, D21S112, D6S22, and D11S129, and further are capable of initiating the synthesis of an extension product including at least a portion of the VNTR region in the presence of the appropriate nucleoside triphosphates and a suitable polymerization agent. These primers are not capable of hybridizing to only the VNTR region under Polymerase Chain Reaction (hereinafter PCR) extension conditions.

The oligonucleotide primers preferably are provided in pairs adapted to interact with a common segment of DNA to amplify a VNTR region of a locus. A first oligonucleotide primer of the pair has a sequence complementary with at least a portion of a VNTR flanking region at one end of a VNTR region of a first strand of a locus. The second oligonucleotide primer of the pair has a sequence complementary with at least a portion of the VNTR flanking region of a second strand of the locus complementary to the first strand at the opposite end of the VNTR region. The pair of primers are

non-homologous to and non-complementary with one another. Preferably, the primers are not capable of hybridizing to only VNTR regions under PCR extension conditions.

In one aspect of the invention, a method for determining the genetic identity between two samples of DNA is provided. A first sample of DNA is treated to produce fragments of DNA, the fragments having at least one VNTR region of the locus selected from the group consisting of D18S17, D1S47, D20S15, D21S112, D6S22, and D11S129 and the at least one VNTR region being present in its entirety as a contiguous piece of DNA. The DNA fragments, or an amplified product thereof, are separated based at least in part upon the size of the VNTR regions thereby producing a fingerprint of the first sample. The foregoing procedure is repeated with a second sample of DNA, and the fingerprint of the first sample and second sample of DNA are compared to establish or exclude identity of the DNA samples.

In one preferred method for carrying out the invention, a Southern Blot Assay and the probes of the invention are used to create the fingerprint. A first sample of DNA is digested with enzymes to produce fragments of DNA. The digested fragments are separated in a gel under conditions which allow the fragments of DNA to move to positions within the gel based upon their size, thereby creating a pattern of bands. The DNA within the gel then maybe transferred to a substrate maintaining the pattern of bands. The DNA on the substrate then can be

contacted with the labelled oligonucleotide probes of the invention to create the fingerprint. Alternatively, the DNA can be dried within the gel and contacted directly with an oligonucleotide probe(s). The steps are repeated with a second sample of DNA, and the fingerprints created are compared.

In another preferred method of the invention, PCR is employed using primers of the invention to create the fingerprints. First, at least one VNTR region of a first sample of DNA is amplified, the VNTR region being within a locus selected from the group consisting of D18S17, D1S47, D20S15, D21S112, D6S22, and D11S129. The amplified DNA then is separated from other DNA in the sample based at least in part upon the size of the amplified DNA, and a fingerprint is created. Preferably the DNA is separated in a gel based upon size and the fingerprint is created by staining the DNA in the gel. The steps are carried out with a second sample of DNA to create a second fingerprint, and the two fingerprints are compared to establish or exclude identity.

Preferably, the foregoing methods of the invention are carried out in connection with at least two different VNTR loci, and most preferably three or more.

It is an object of this invention to identify and provide sufficient DNA sequence information for several highly polymorphic human DNA loci to permit discrimination of different alleles of these loci in different individuals.

It is another object of this invention to provide sufficient DNA sequence information for several highly polymorphic human DNA loci to permit the design of oligonucleotide primers or oligonucleotide probes suitable for detection of various alleles at these loci by DNA hybridization methods.

It is a further object of this invention to provide sufficient DNA sequence information for several highly polymorphic human DNA loci to permit the design of a variety of oligonucleotide primers or probes suitable for identification of these loci by using methods such as Southern Blot and PCR.

BRIEF DESCRIPTION OF THE DRAWING

The figure is a photograph showing the gel electrophoresis separation of five DNA samples, portions of which were amplified using primers capable of hybridizing with regions which flank the VNTR region of locus D20S15.

DETAILED DESCRIPTION

This invention relates to probes and primers capable of hybridizing with various regions within or flanking a particular group of VNTR loci and methods of using these probes or primers for determining identity.

The term "oligonucleotide" as used herein in referring to primers, probes and extension products refers to a molecule comprised of two or more

deoxyribonucleotides or ribonucleotides, synthetic or natural, and preferably more than three. The exact size of the molecule may vary according to its particular application.

The term "probe" refers to an oligonucleotide capable of hybridizing to a strand of DNA in connection with determining the existence and location of the strand of DNA. The probes of the invention typically are labelled, isotopically or non-isotopically. For example, labelled nucleoside triphosphates may be used in constructing the probes or the probes may be labelled post-construction. The label may be, for example, a radioactive label, an enzyme, or a fluorescent chromophore.

The term "primer" refers to an oligonucleotide which, when hybridized to a strand of DNA including a complementary or substantially complementary sequence, is capable of initiating the synthesis of an extension product in the presence of the appropriate nucleoside triphosphates and a suitable polymerization agent. Preferably, the primer is an oligoribonucleotide and most preferably is a oligodeoxyribonucleotide. The primer, however, may be other than a ribonucleotide or deoxyribonucleotide. The primer must be sufficiently long to prime the synthesis of an extension product. The exact length of the primer will depend upon many factors, including the degree of specificity of hybridization required, temperature of the annealing and extension reactions and the source and structure of the primer.

The term "capable of hybridizing to" refers to all methods of sequence specific pairing between the probe or primer and a strand of DNA. It includes situations in which there is complete complementation between the molecules hybridizing to one another, and situations when there is less than complete complementation.

The term "corresponding to" when used in connection with primers and DNA sequences shall mean a primer homologous or substantially homologous to a DNA sequence or a primer complementary or substantially complementary to a DNA sequence.

The term "complementary" when referring to two oligonucleotides or an oligonucleotide and a DNA sequence shall mean sequences which are completely complementary as well as those sequences which are substantially complementary (i.e. less than complete complementation -- some mismatches of base pairs). By substantially complementary, it is meant that the oligonucleotides are capable of hybridizing to one another to perform their intended functions in the invention under appropriate conditions.

The term "homologous" when referring to two oligonucleotides or an oligonucleotide and a DNA sequence shall mean sequences which are identical or which are substantially identical. By substantially identical, it is meant that the oligonucleotide may have some mismatches but is still is capable of performing its intended function in the invention under the appropriate conditions.

The term "fingerprint" refers to a pattern derived from separated DNA fragments prepared from a source of DNA. An example of such a fingerprint is a pattern produced by separating fragmented DNA based upon size within a gel using gel electrophoresis.

The oligonucleotide primers and probes of the invention may be prepared using any method, such as, for example, methods using phosphotriesters and phosphodiesters well known to those skilled in the art. In one such automated embodiment, diethylphosphoramidites are used as starting materials and may be synthesized as described by Beaucage and Caruthers 1981, Tetrahedron Lett. 22: 1859-1862. One method for synthesizing oligonucleotides on a modified solid support is described in U. S. Patents 4,458,066 and 4,500,707. It also is possible to use primers or probes which have been isolated from a biological source (such as restriction endonuclease digest of plasmid DNA).

The samples of DNA used in the methods of this invention can be from any source of nucleic acid, in purified or non-purified form. The DNA can be extracted, for example, from blood or tissue material using a variety of conventional techniques such as those described by Maniatis et al. Molecular Cloning: A Laboratory Manual, (New York: "Spring Harbor Laboratory, 1982) pp. 280-281.

The degree of certainty with which two DNA samples can be said to be from the same individual depends on the heterozygosity of the particular

polymorphisms under investigation and on the distribution of particular alleles within different populations (E.S. Lander, 1989, Nature 339: 501-505). For example, some alleles may be very common in certain isolated populations. In that case, the presence of the same allele in two different DNA samples would not indicate a high probability that those two DNA samples were from the same individual (the presence of different alleles in the two samples, however, would indicate a high probability of exclusion of identity). A database of information on allele frequencies can be obtained. In general, a database with information from at least 1000 chromosomes for each loci for each ethnic group would be ideal for DNA typing studies. Databases of allele frequency distributions in populations are gathered by standard methods, and recently, a preliminary database for five VNTR probes used in paternity studies was reported (Balazs et al, 1989, Am. J. Hum. Genet. 44: 182-190; Odelberg et al, 1989, Genomics 5, 915-924).
VNTR polymorphisms, in general, exhibit very high heterozygosities because very many different alleles occur. A difference of one repeat unit constitutes a different allele. (Balazs et al, 1989, supra).

The DNA sequences of this invention are useful for the application of rapid, sensitive methods for the determination of identity or exclusion of identity between the genetic material in two or more

samples. These DNA sequences permit both the synthesis of labeled oligonucleotide probes to identify VNTR alleles in Southern Blot fingerprints and, alternatively, the synthesis of PCR primers so that VNTR alleles can be analyzed without the need for labeled probes and Southern blot fingerprints.

Analysis of the genetic loci described by the DNA sequences herein can establish identity or exclude identity between DNA samples with a high degree of certainty. For example, because the loci describe here exhibit very high heterogosities a combination of the data resulting from the use of all of the probes described here on actual DNA samples should provide an average probability of exclusion of identity in excess of 99.95% and an average probability of identity of better than 9.4 x 10^{-9}. Excellent probabilities of exclusion or identity suitable for many applications may also be obtained by using various subsets of the probes described here. Actual probabilities of identity and of exclusion of identity will depend on the distribution of alleles in a specific population as determined by testing the DNA of many individuals. By compiling such databases of allele distribution analysis of the alleles at the loci described here in any two test samples will provide quantitative estimates of identity or lack of identity.

Nucleic acid probes capable of hybridizing to over 180 different loci were described by Donis-Keller et al., cited supra, the contents of

which are incorporated herein by reference. The DNA probes CRI-L159, CRI-L336, CRI-L355, CRI-L427, CRI-L1077 and CRI-R365 were selected from this large group of probes as potentially including VNTR regions. These probes were prepared and isolated using the techniques described by Donis-Keller et al. Subsequent to selection, the probes were sequenced using conventional techniques. The DNA sequences of the cloned loci were determined by the dideoxy chain-terminating method (Chen & Seeburg, 1985, DNA 4: 165-170; Tabor & Richardson, 1987, Proc. Nat. Acad. Sci. USA 84: 4767-4771). From sequence analysis of these probes, the DNA sequences of seven repeated VNTR regions and the surrounding unique regions were identified.

A variety of methods may be used to identify the alleles at the loci defined in this invention. The Southern blot is a reliable approach to measuring VNTR lengths and thereby identify alleles.

In a Southern Blot Method, a first DNA sample is digested with enzymes to produce fragments of DNA. These enzymes are capable of cutting the sample of DNA into fragments containing VNTR regions in their entirety as contiguous pieces of DNA. Examples of such enzymes include AluI, PstI, and HaeIII. The selection of enzymes depends upon the loci being examined. Table 1 shows the preferred enzymes for each locus and/or type of probe.

Table 1

Locus	Detected by Probe derived from:	Enzymes
D18S17	CRI-L159-1	AluI
D18S17	CRI-L159-2	PstI or AluI
D1S47	CRI-L336	PstI
D20S15	CRI-L355	HaeIII
D21S112	CRI-L427	AluI
D6S22	CRI-L1077	PstI or HaeIII
D11S129	CRI-R365	HaeIII or AluI

The digested fragments are separated in a gel under conditions which allow the DNA fragments to be separated based upon size. The gel is selected based upon such factors as the size of DNA being separated and ability to withstand conditions necessary for separation. Examples of gels include agarose and polyacrylamide gels. The conditions, which allow the DNA fragments to be separated, can be determined by one of ordinary skill in the art.

The DNA within the gel then is transferred to a substrate using conventional techniques, e.g. blotting or the gel can be dried andthe DNA can be contacted directly with a probe while it is within the dried gel (Ali and Wallace Nucleic Acid Research Vol. 16, No. 17 (1988), pp. 8487-8496. The substrate is made of a substance which is capable of maintaining the pattern of bands of DNA transferred from the gel while not interfering in the hybridization process. Examples of such substrates include nitrocellulose filters and nylon membranes.

The DNA on the substrate is then contacted with a labelled oligonucleotide probe to create a fingerprint of the sample. The DNA sequences provided in this invention permit the use of synthetic DNA segments as probes. Such synthetic DNA is designed to be long enough to provide specificity of hybridization to the desired region, but short enough to be synthesized economically. The probe can be labelled by a variety of means. For example, it can be labeled at its 5' end by T4 polynucleotide kinase (Maniatis, Fritsch, & Sambrook, "Molecular Cloning — a Laboratory Manual", Cold Spring Harbor Press, Cold Spring Harbor, N.Y., pp. 122-127), or throughout most of its length by amplification according to the PCR method in the presence of labeled dNTPs as described in U. S. patents 4,683,195 and 4,683,202. The contents of both patents are hereby incorporated by reference. Preferably, the probe is labeled by a nonisotopic method, thereby providing long shelf-life and stability. For example, such an oligonucleotide could be labeled with an enzyme such as alkaline phosphatase (Jablonski et al, 1986, Nucl. Acids Res. 14: 6115-6128). After hybridization to the DNA sample, its presence would be detected by action of the enzyme on a substrate to produce a signal. Such substrates provide visual signal by absorption of certain frequencies of visible light (Edman et al, 1988, Nucl. Acids Res. 16: 6235), or they expose film by emission of light

in the process of chemiluminescence (Bronstein & McGrath, 1989, <u>Nature</u> 338: 599-600). The latter method provides high sensitivity for detection of very limited amounts of sample. Besides sensitivity and convenience of long shelf-life, synthetic oligonucleotide probes also contain no contaminating labelled vector or bacterial DNA sequences. Thus, they can be used to analyze DNA samples which are contaminated by bacteria.

Several parameters can be adjusted to insure that a single locus is detected by a particular oligonucleotide probe. For example, the length and specificity of the probe may be varied, the probe may consist of a mixture of probes which vary in sequence at one or a few nucleotide positions, and the stringency of hybridization may be varied, for example, by altering temperature and formamide concentrations as is known in the art.

The oligonucleotide probes of the invention are homologous or complementary to only a portion (not all) of any of the sequences of Examples 1-7. This portion may correspond to only a VNTR region, only a flanking region or both a flanking region and a VNTR region contiguous with one another.

The probes may also be of a size corresponding to a single repetitive sequence in a VNTR region. This type of probe may hybridize in multiple locations in the VNTR region providing increased

sensitivity because of the increased number of labelled sites. The probe in one preferred embodiment may correspond to a portion of or to the whole of any single tandem repeat of a VNTR region. Alternatively, the probe may correspond to a contiguous region of two tandem repeats, i.e., the probe would hybridize to and overlap two adjacent single repeats. The probe in another preferred embodiment is of a size corresponding to more than one repetitive sequence, but preferably of a size corresponding to less than five repetitive sequences.

The oligonucleotide probes are of a size large enough to provide the required specifity but are generally of a length which can be synthesized economically. The preferred probes are 15-60bp in length, most preferred 18-40bp in length. When using a panel or set of probes, the probes should be sufficiently non-homologous to each other so that one probe will not hybridize to two different loci. One of ordinary skill in the art will know how to construct probes based on the information provided herein.

An entirely different approach to measure these VNTR patterns by-passes the Southern blot altogether and makes use of the PCR method (US patents 4,683,195 and 4,683,202, the contents of which are hereby incorporated by reference). By this approach, the DNA sequence of some portion of the unique DNA on either side of the repeated VNTR region is determined. For example, by using the

unique flanking sequences provided in this invention, DNA primers can be designed and synthesized for use in amplifying the repeated region between the two primers. By amplifying this region of the DNA of each individual, separating the amplified product according to size on a polyacrylamide gel or an agarose gel, and staining the gel to reveal the position of the amplified DNA, it is possible to determine the size of the repeated region between the primers (Boerwinkle et al. 1989, Proc. Nat'l. Acad. Sci. USA 86: 212-216; Horn et al, 1989, Nucl. Acids Res. 17: 2140). Thus, by this approach, no label and no Southern blot transfer of the gel are needed. However, two specific primers are needed for amplification of each VNTR region desired.

While probes hybridizing to VNTR containing loci in the human genome have been described, the unique flanking sequences necessary for design of PCR primers are known in only a very few cases (Jarman et al. 1986, supra; Boerwinkle et al. 1989, supra; Horn et al. 1989, supra). Here, DNA sequences suitable for application of this method at seven new genetic loci are provided. A pair of primers may be used to amplify the VNTR region of a single VNTR locus or two or more pairs may be used to amplify (simultaneously or otherwise) the VNTR regions of two or more loci.

Development of a working PCR is basically as specified in the U. S. patents incorporated by reference above. Briefly, important variables to

optimize are the temperatures of the annealing and polymerization reactions and the length of time allowed for the polymerization reaction. The two primers for a specific PCR are chosen so that they have similar annealing temperatures. Reaction times are long enough to provide full extension but not so long as to allow excessive mispriming and generation of artifactual bands.

In order to apply the PCR amplification method to analysis of alleles at the loci described in this invention, it is important that the PCR primers hybridize to unique single-copy DNA sequences flanking the VNTR repeated regions. Since recent studies have shown that some VNTR repeats are themselves found within other moderately repetitive DNA regions (Armour et al., 1989, Nucl. Acid. Res. 17: 4925-4935), it may be necessary in some cases to test PCR primers homologous to several different flanking DNA sequences to insure that a primer is selected from a unique region and that a single PCR amplified product is produced.

Preferably, the unique single copy (flanking region) amplified along with the VNTR region should be as small as possible, or at least of a size that will not interfere with the detection of the number of repeat sequences in the VNTR regions of compared DNA samples. For example, if the unique sequence amplified is 1000 base pairs, detecting the difference in the VNTR regions of two samples of DNA, one with a VNTR size of 200 and another with a

VNTR size of 240, would be difficult. In most cases, the unique single copy DNA will comprise less than 50 percent of the entire portion amplified. In a preferred embodiment, the unique sequence amplified is less than 100 base pairs and most preferably between 30 and 60 base pairs. This provides sufficient priming template at either end of the VNTR regions (primers typically being of a size between 15 and 30 nucleotides), yet is small enough so as to not interfere with the ability to distinguish VNTR regions which are close in size.

The limit on the size of the unique sequence amplified will depend upon the size of the repetitive sequence tested and upon the frequency distribution of the number of repeats in the VNTR locus of the populations tested. The probes of the invention may be used to determine this frequency distribution in the population. The probes can be used to isolate the VNTR locus of various individuals within the population. These isolated VNTR regions then can be sequenced to determine the number of the various repeats. This information can be compiled in a database to form a profile representative of the frequency distribution of the repeats in the population. The upper limit on the size of the unique sequence amplified then could be determined by one of ordinary skill in the art.

The pairs of oligonucleotide primers preferably are adapted to interact with a common segment of DNA containing a VNTR region within a locus selected

from the group consisting of D18S17, D1S47, D20S15, D21S112, D6S22, and D11S129. One of the pair has a sequence complementary with at least a portion of the flanking region at one end of the VNTR region of a first DNA strand of the selected locus. The other of the pair has a sequence complementary to at least a portion of the flanking region of a second DNA strand (complementary to the first strand) at the opposite end of the VNTR region of the selected locus. The pair of primers should be nonhomologous and noncomplementary with each other.

The primers preferably do not hybridize to only the VNTR regions under extension conditions. While the primers may be complementary to a contiguous flanking region and VNTR region, there preferably is insufficient complementation with the VNTR region to permit extension priming at other than the ends of the VNTR regions (i.e. no priming within a VNTR region under PCR extension conditions). Thus, the primers are preferably nonhomologous and substantially noncomplementary to the nucleotide sequences of the VNTR regions of Examples 1-7, below. Most preferably the primers are complementary or homologous to at least a portion of the flanking sequences of Examples 1-7.

The invention now will be illustrated by the following examples.

EXAMPLE 1

The following DNA sequence includes flanking regions around the VNTR locus D18S17 detected by probe CRI-L159-1 on human chromosome 18 and seven copies (denoted 1-7; additional unsequenced copies are present between the sequenced copies 3 and 4 -- this is indicated by a dashed line) of the repeated region. Repeated units are aligned to maximize sequence homology

```
PstI
5'CTGCAGCTGC CCCAGGGTTC TCCAGCCATG GGCTCCATGC TCA

1      CCCGGCCGCA CCAGGTTGAT GGGGGGCGTG TGCCCGCTCT CGCCTTCC
2      CCCGGCCGTA CCAGGTTGAT GGGGGGCGTG TGCCCGCTCT CGCCTTCC
3      CCCAGCGGTA CCAGGTTCAT GGGGGGCGTG TGCCCGCTCT CGCCTTCC
       ---------- ---------- ---------- ---------- --------
4      CCCGGCGGTA CCAGGTTCAT GGGGGGCGTG TGCCCGCTCT CGCCTTCC
5      CCCGGCGGTA CCAGGTCGAT GGGGGGCGTG TGCCCGCTCT CGCCTTCC
6      CCCGGCGGTA CCAGGTCGAT GGGGGGCGTG TGCCCGCTCT CGCCTTCC
7      CCCGGCCACA CCAGGTTGAT GGGGGGCGTG TGCCCGCTCT CGCCTTTC

TCACTGTCCC TCGCGGTCGC TGGCTGAGAC CAGGTGGCAG CGAGTTTTCT CGAGAGGAAC
TGAAACGCGT GAGTCGACGT CTGAGCCTCA GCCACACGCT GCCTGGCGCA CAGGTTTGGA
CGCGGCCTCT GGTGGCCGCC TCACCTCGGG CACGTCACAG GGTGGGCTCC CTGAGGAAGC
AGCTCCGGGC CTGGGGCTGG ACGGGCTCTT GGCGTTGGGG AGCTGCCTCT TCAGCGGCCT
CCGGAGCACC CGGCCCCGCA GCCCGGCCCG CCCTGCCGAC AGCAGGCTCC GTCTCCGGGT
GGCGTGCGGT GCGATTTTTA CGCGTTGAAA CCCCCACGCC GCGGTCCCAC GGCACCAGGC
ACTGACCCCG ATGAATGATT TGCACGGCTG AGTCCGCTGC TCCATCCATC ACGCGGTAGC
CGCCGTCTGC GACGGTAACG ACGTTGGATG GCGGCACACA GGCTCTTTCA ATCATCCTCA
GCCGGGGGGA AGGCCTCAGA CCTGGGCGCC GGGCATGAAT TC3'
                                          EcoRI
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EXAMPLE 2

The following DNA sequence includes flanking regions around the VNTR locus D18S17 detected by probe CRI-L159-2 on human chromosome 18 and seven copies (denoted 1-7; additional unsequenced copies are present between the sequenced copies 4 and 5 -- this is indicated by a dashed line) of the repeated region. Repeated units are aligned to maximize sequence homology.

```
5'AACCGCTCTT TATAAGGAGT CCTTTTTCTT GCATTAACAA AATGCTCAAA ACTCAGTTGA
AGAAAAGTAA AAAGCACGTG GCATTGCTAG GGTCTCCCAG CCCCTCCCTA AACACACAGC
ATTCTACGGA GGCACGTTTC ACACAGGTAA TGCACCCTTG AGAAGGGCAC AAAATTCAGT
GATTTTTGGT AAAGGTGCCC AGTTGTGCCA CCATCACTGC AGGTGTCAGA ACA
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1  CTTCCATCAC CCAAGGGGCT CGTCCACCAC CTCTTCCCAC AAAGCGGTCT CCTGCCT
2  CTTCCCTCAC CCAAGGGCCT CCAGCACCAC CTCCTCCCAC GCAGCAGTCT CCTGCCT
3  CTTCCCTCAC CCAAGGGCCT CCAGCACCAC CTCCTCCCAC ACAGCAGTCT CCTGCCT
4  CTTCCCTCAC CCAAGGGTCT CATCCACCAC CTCCTCCCAC ACAGCAGTCT CCTGCCT
   ---------- ---------- ---------- ---------- ---------- -------
5  CTTCCCTCAC CCAAGGGCCT CCAGCACCAC CTCCTCCCAC GCAGCAGTCT CCTGCCT
6  CTTCCCTCAC CCAAGGGCCT CCAGCACCAC CTCCTCCCAC GCAGCAGTCT CCTGCCT
7  CTTCCCTCAC CCAAGGGCCT CCAGCACCAC CTCCTCCCAC GCAGCAGTCT CCTGTCT

CCAGAGCTGC GCCTTTTGTG GATGTCTTGC AGGCAAGCCT CACTTCACTG TGCTTCAGAC
ACTGCTTTTC ACAAACGGGA GGTCTTTGGC AACCCTGCAC CAAGCACGTC TGTGGGCACC
ACGTTTCCAA CAGCAGGTGC CCACTTCATA TCTCTGTGTC ACGTTTCAAT AATGCTCATG
ATGTTCCAAG CTTTCTCATT GCTATCATAT CTGCTGTGGA GATCTGTGAT CTTTGGGGTT
ACTGTTGTCA TTGTTTTGGG GTGCCACGAA CTGTGCCCAT CTAAGATGAA GTGAATGGAT
AAGAGCTGGG TGCATCCTGA CTGCTCCACT GACCAGCCAT TCTCTATCTC TCTCCCTGGG
CTCAGGCCTC CCTATTCCCT GAGACACAAC CATACTGACA TTAGGCCAAT TAAGAACCCT
ACGACAGCCT CCAAGTTTTC AAGTAAAAGT TCAAGGGCAA GGAAAGGTCA CACGTCTCTC
ACTTCAAAAC TAGGAATGAT TAAATTTGGT GAGGAACACC CGTCGAAAGC CGAGACAGGC
TGACAGCTCA GCCTCCCGCG CCAGTCCAAC TGTGAATGCA GCGGAAAACT CTAGACAGAC
AGAAGTGAAA ACCGCTGCCC TAGTGAACAA CACCGATGAT AAGAGGGCGG AACAGCCTTA
TTGCTGATAC AGAGAAAGTC CGAGTGATCT GGACAGAAGA TTGAACCAGC CACAACACTC
CCTTAAGCCA AAGCCTAATC CAGACCAAGA CCCTCGCTCT CTCTGTCCTA CGAAGGCTGA
GAGATGAGGA AGCTGCAG 3'
              PstI
```

EXAMPLE 3

The following DNA sequence includes flanking regions around the VNTR locus D1S47 detected by probe CRI-L336 on human chromosome 1 and twelve copies (denoted 1-12; additional unsequenced copies are present between sequenced copies 5 and 6 -- this is indicated by a dashed line) of the repeated region. Repeated units are aligned to maximize sequence homology.

```
PstI
5'CTGCAGATGG GCATTGCTGC ACCCAGCTTG GTTAGAGGGT TTTTAAGCCT AGGGGGTGCA
TTTGTTTCCT AGTACCACAA GCAGGGTGGC TTGAAACAGC ATTATTTATT CTCTCATGGA
TCAGGAAGCC AGAAGGCAAG GTGTGGGCAG GGTTGGTTCC CTCTGGAGAC CGAGGTAGCA
TGTGTTCCAG GCCTCGCTCC TGGTTCCTGG TGGCTGCCCG TAGTGTTCCT TGGCTTGTAG
AAACACCCCC CCGAGATTCT GCCCCCATCG CCACATGGCC TTTTCTGTGT CTGTCTCCTC
TTTTTATATG GACACTTGTC ATTGGATTTA GGGCCCATCC TAATCCAGGA TGGCCTCATC
GCAGGATTTC CACCTTAATT ACATCTGCAA AAACCCTTTT GCCAAATAAT TCCACATCGA
CAGGTAACTG GGATTATGAC TTGGGTGGAT CTTTCAGGGA ACACAGTCCA CCCCACACAA
TAGGTTTCTA CCCCCGAGTC CCACCTCCAG CCCCGTCTCC CTCCAGAGTC TGGCTGCTTC
TTCCTCATCA GACAGTTCCT TCCCTCTGTA GGGATTGCAT ACAAGTGTT
```

```
1  GCTG GCTGA GGGGTGAGGG TGGAGTGGGG GGATGTGGGA
2  GCTGAGCTGA GGGATGAGGG TGCAGGCAGA  GATCTGGGA
3  GCTGAGCTAA GGGGTGAGGG TGGAGTAGGG GGACGCGGGG
4  GCTGAGCTGA GGGGTGAGGG TAGAGT GGG GGACATGGAA
5  GCTGAGCTGA GGGGTGAGGG TAGAG
   ---------- ---------- ---------- ----------
                               TGGGG GGATGTGGGA
6  GCTGAGCTGA GGGGTGAGGG TGGAGTAGGG GGACGTGTGA
7  GCTGAGCTGA GGGGTGGGGG TGGAGTTGGG GGATGTGGGA
8  TCTGAGCTGA GGGGTGAGGG TAGAGT GGG GGACATGGAA
9  GCTGAGCTGA GGGGTGAGGG TAGAGTGGGG GGATGTGGGA
10 GCTGAGCTGA GGAGTGAGGG TGAACT GGG GGATGTGGGA
11 GCTGAGCTGA GGGGTGGGGG TGGAGTTGTG GGTTGTGTGA
12 GCTGAGCTGA GGGGTGAGGG TGGAG GCGG GGACGTGGCA
   GCCCAGCTGA GGG 3'

CCTTGGAGCA CACCTGGTAG GAAGCCAGCC TGTGGACCTG GAGCCGACTG AGGGTCCCCC
CTACCCCCCA TGAGTAGGAG TGGGATCC
                        BamHI
```

EXAMPLE 4

The following DNA sequence includes flanking regions around the VNTR locus D20S15 detected by probe CRI-L355 on human chromosome 20 and thirteer copies (denoted 1-13; additional unsequenced copies are present between sequenced copies 8 and 9 -- this is indicated by a dashed line) of the repeated region. Repeated units are aligned to maximize sequence homology.

```
5'ATAAAAATCA TACAAAGTAT ATTTTAAAAG ATCAAATTAA ACTAGAAAAT AACAAAGATA
TCTGAAAATC TCTAAATATT TGGAAACTAA TAATGTACTT GTCTGTAAAA TGTGGATAAA
ATGGAATTAT CTGAGAATTA AAATTCTTTT GAACTGAATG ACAATAAAAA TACAGCATAT
CAAAATGTAT GGGATGCAGC TAAAGCAGTG CTCAGAGGAC AATCTATACT ACTAGAGGCT
TATATTAGAA AAAAAAATTT AAATCAATAA CCTAAGCTTT CACCATTAGA GAAGGAATT

1  TCCTCACTCA GGACCCTCTG CGGATGTCGC CTCCA
2  TCCTCA TCA GGACCCTCCA TGG TGTCAC CTCCA
3  TCCTCACTCA GGACACTCCA TGG TGTCAC CTCCA
4  TCCTTACTCA GGACCCTCCA TGG TGTCAC CGCCA
5  TCCTCACTCA GGACCCTCCA TGAGTGCCAC CTCCA
6  TCCTCAC CA GGATCCCCTG TAAGTGTCAC CTCCA
7  TCCTCAC CA GGACCCTCCA TGAGTGTCAC CTCCA
8  TCCTCACTCA GGACCCTCCA T
   ---------- ---------- -
9  TCCTCAC CA GGACCCTCCA TGAGTGTCAC CTCCA
10 TCCTCACTCA GGACCCTCCA TG GTGTCAC CTCCA
```

11 TCCTCACTCA GGACCCTCCG TGAGTGCCAG CTCTG
12 TCTACCCTCG GGACCCTCCA TGAGTGTCTC CTCTA
13 TCTACCCTAA GGACCCTCTG TGAATGTCCT CGCCA
TCCATACTC

CCCCCTCTTA GACCCAGACT GTTTCAGGGT TGGACACTGA GCTGTGGCCC CTGAGCCTAG
CTCTTCTCCG AGCTTCTCTT GGGCACCTCT CTCTCAGACT AGGCTCAGCT CCAACACCAG
TTCCCAGAGC CCTTCCTGTT CACTGTCTGG TGTCTCACAC TCACACCTGC TCACATGCAC
ACTGACTCAC TCACACTCAA ACTCACATCC ACAGTTATAG AGTTCACTGC TTTCCTCACA
TCCTTCTGCA GTCAGGTGGC CACAGTCACC TTCTCCCACA CAGCCTCACA CACTCATGCT
CCCAGACACG TTCACAGATA TACACAAACT CATTGACACA CTGGCAAACT CAATACTGTG
CACACCTGCT CTCAGGCACA CACTCACACA CTCATCCACA CAGAGACACT CACTCCTGTG
CAGGTGCACA CATGACACGC CCCCCCACCC CTCCCTTCCG GCACCCCATG TTCCCTGTCT
CAGGGCTCAC ACTTCATAAG CACTCATGGA AGTTGCTACT GCAGGGTGGA TGCCAAGACA
GGGAGCCTGC ACGAACAGGT TCACAGCATT TGTTGTTTAT GTGGCTGTTG CTTGTCCTTC
ACTGAGGTCC AGCATTTAGA GGGCAGGGAC TCTGTCTCTG GGCATCTCT TTATTCCAGG
ACACAGCAAG TACCTGCCAC ATCTAGGAAC TCAGTAAATA TTTCTTGTTT AATAAGAGAG
GGAGGAAGCA CAGGCTATAG GATACAAAAA AAAAGAAGAG GATGGCAAAG AAGGACGGGT
GCTGAGGAAG ACAGAGAGAT GGACCGTGTT GAGCCTGCGA GGTCTTGGCG GGAGCTGTCC
TGGGCGTGGT TGTTCTCTGA TACAGTCTAA TTTCGATGTG GCCTCGGTGT GGCATACTAT
GGGACCACAG CTTCCCCATC AGGCTGCCTT ACTGAGGTGG AACTCCACAA CCTTGATTTA
ATAGACAGAA GCTGCATCTG CTCCCCACAA GTTACTAGCC CACACGGGCA CTGCTCAGAG
TTAAGAATGT GTTTCCATGG TGACCTTCTG GTAATTAACT TTTTGGAAGG TGTCCAAGAC
AGACCAGCCT CCTCCAGAAT TC 3'
EcoRI

EXAMPLE 5

The following DNA sequence includes flanking regions around the VNT[illegible] locus D21S112 detected by probe CRI-L427 on human chromosome 21 and fourteen copies (denoted 1-14; additional unsequenced copies are present between sequenced copies 6 and 7 -- this is indicated by a dashed line) [illegible] the repeated region. Repeated units are aligned to maximize sequence homology.

5'CCACCAGCAA GCCCCCAGTG GACGCTGAGG CTGGTGGTGC CTGCCAGCCA GTGTGGGTCC
CTGATCGGCA AAGGAGGCTC CAAGATCAAG GAGATCAGGG AGGTAACAGG ACCTTCCCAG
CCTGGGCCGC TGCGGAGCCT CTAGGCGGGC TGCGGGTGGT GGCCACAGGC CAGGCAGCCT
TCCTGAGCCT TGTCCCTGCT GTCTGCAAGC CCAATGCTGG CCACGCAGAC CCCACAGCTC
AAAGTGCGAG ACAGGAACAC AGGACCCATG AGCAAGCACA ACTGCACTGC AG

1 GGACCCAGCC CACCCACT GCCCAGATAC
2 GGACCCGGC CACCCACT GCCCAGATAC
3 AGACCTGGCC CACCCACT GCCCAGATAC
4 GGACCCCCCC CACCCACT GCCCAGATAC

```
5    GGACCCCCCC CCCACCCACT GCCCAGATAC
6    GGACCCGGCC   CACCCACT GCCCAGATAC
     GG
     ---------- ---------- ----------
7      ACCCGGC    CACCCACT GCCCAGATAC
8    AGAGCCCGGC  CCACCCACT GCCCAGATAT
9    GGACCCCCCC   AACCCACT TCCCAGATAC
10   GGACCCCCCC  ACACACACT TCCCAGATAC
11   GGACCCCCCT   CACCTGCT TCCCAAATAT
12   GGACCTGGCC   CACCCACT GCCCAGATAT
13   GGACCCCCCC   CAC CACT GCCCAGATAC
14   GGACCCCCCC   CAC CACT GCCCAGATAT
     GGACC
```

TGGCATGAAT TCCAGCTGAG CGCCGGTCGC TACCATTACC AGTTGGTCTG GTGTCAAAAA
TAATAATAAG GCCAGGCCAT GTCTGCCATT TCGCGTAAGG AAATCCATTG TACTGCCGGA
CCACCGACTG TGAGCCACTC CGGCCATGGG TACGACTGAC CTGCTTACTG ATTTGTAAAA
CCGGTCCCGG CCATCACGCT CA 3'

EXAMPLE 6

The following DNA sequence includes flanking regions around the VNTR locus D6S22 detected by probe CRI-L1077 on human chromosome 6 and ten copies of the repeated region (denoted 1-10; this locus in the individual from which this clone was derived carried only these ten repeat copies). Repeated units are aligned to maximize sequence homology.

5'CTGTTCCTGA GCCCTGTGGA TGGTTCAGAC GTCCCCATCC ACATGAGGGT CATGTGATGC
GGCTTTAATT TCCACCTGGC AACCCTGGAA GCCCACGTGT GTCTCAGGAA TGCATGGCTT
CCATCTCCTC TACAGCCACA GGCAACAGGT CTACCTAGGG CAT

```
1    GTGTGGGCAG CACAGGGTGC ATGGTCACCT GCAGTGGATG
2    GTGTGGGCAG CACGGGGTGC GTGGTCTCCT GCGGTGGGTG
3    GTGTGGGCGG CACGGGGTGC GTGGTCTCCT GCGGTGGGTG
4    GTGTGGGCGG CACGGGGTGC GTGGTCTCCT GCGGTGGGTG
5    GTGTGGGCGG CACGGGGTGC GTGGTCGCCT GCGGTGGGTG
6    GTGTGGGCGG CACGGGGTGC GTGGTCGCCT GCGGTGGGTG
7    GTGTGGGCGG CACGGGGTGC GTGGTCTCCT GCGGTGGGTG
8    GTGTGGGCGG CACGGGGTGC GTGGTCGCCT GCGGTGGGTG
9    GTGTGGGCGG CACGGGGTGC GTGGTCGCCT GTGGTGGGTG
10   GTGTGGGCGG CACGGGGTGC GTGGTCGCCT GTGGTGGGTG
```

TGCGGGGCCT TCTGTGAGGC AGGACAGGCT CTACATGCAC TTCGGCTTCT TGGCAGATCT
GAGCGAATGA GAACTGCCCT GGGCTTCGCT CCCGTTTAAC TTTGCATGAA GAAGAATGTT
CTCCACTCTG ACTTTAAGCC AAGTGCTTGT CTAAGTGCTT CTCTGACAGG GAGACAACAC

TCCTTCCCCG GAGCCTTCCA CTGGGCCTGG ACATCTCCCG TCTCTGGGCT CTGAGTTAAC
TTTGTGATGC CAGCTCTTTG TGGGGTGTGC GCACGGCTGC AGGTGAGGAG ACCTGTGAGA
GTCGGAAGCC AGCTGCCACC CCAAATGATA AGCACATTGT AGCTGTCTCT GCATGGCTTC
GTTGCATGAG GAAAGTCACT ACTGTCAATG CAGGGCTGCT GGTCTCCACC TCGGAACGTG
GCAGTGCACA ATCCATCACT GCCCTGTGTT CGCAGGTCAC AGAGAAAAGA AATGCTTTCT
CTATTCCTTC TCGCAGTTAG TATTTGTGA TTATAGAAAA 3'

EXAMPLE 7

The following DNA sequence includes flanking regions around the VNTR locus D11S129 detected by probe CRI-R365 on human chromosome 11 and eleven copies of the repeated region (denoted 1-11; this locus in the individual from which this clone was derived carried only these eleven repeat copies. Repeated units are aligned to maximize sequence homology.

PstI
5'CTGCAGGTTT CACAGTCACA CTCACAACTC ATTGTGGAAC TGGGTGTGGG GTGGCCTGAG
ATGTATGTTG CACCAGAGAA CCATGGGCTT TGGAATCTGT GTGAGGGAGT CAGGGACGCC
TGTCGGAGGA GGGGAGACGT GACTGGTTGG TCATGGAAGG CTACAGTTTC GCAGATGGTG
TCGGTGGAGT TTGGGGCAGG GCATTCTGAG CCTGGGAAGG TTTGGGGTGA AGGCCCGGAG
GCTGGCGCGT AGGAGGCTAA AGCAGCAGGG AGGTCTGGGG AGAGTCAGGT GGGGTTGTGT
CTTCCACGTT GCAGGCCGTG CTCACAGATG GTGAAAGACG TTGTGGAAAA GAGAGCTCAC
GAACGAAAAA GGTTGAGTCA AGTC

1 GCTCGGCTGT GCTGTGAGTG TGGGAGGT
2 GCCCGGCTGT GCTGTGGGTG TGAGAGGT
3 GCCCGGCTGT GATGTGAGTG TGGGAGGT
4 GCCCGGCTGT GCTGTGGGTG TGGGAGGT
5 GCCCGGCTGT GCTGTGAGTG TGGGAGGT
6 GCCCGGCTGT GATGTGAGTG TGGGAGGT
7 GCCCGGCTGT GCTGTGAGTG TGGGAGGT
8 GCCCGGCTGT GCTGTGAGTG TGGGAGGT
9 GCTCGGCTGT GCTGTGGGTG TGGGAGGT
10 GCCCGGCTGT GCTGTGGGTG TGGGAGGT
11 GCCCGGCTGT GCTGTGAGTG TGAG

CCCGAGAGCC GCGGAGAACA CGTGGAGCTC TTGCAGTGGC GAACACGTTT CTTCCTAAAG
GAGACACACA GCAATTTTGC TTTCCATGTC CATTCTTGGG ATTGTCTTTC GTGAATGAGT
TAACGGACGC AGAGAAAGCA CTGAGTCTTC CCAGAGGGCC AGTGAGGGGC CAGCTACGCA
AGGCTTCCCA TCCCTGGGGC GCAGCACTCC TGATACCAGG CAGAGGTGTG TCCAGCCCGG
AGCTTGAGCA GCAGCAGCGC CTCCTGACCG ACTCCATGGG GTTGGCCTCA GGGCCTCCCA
GGACCCGCCC AGCCCGGAGA CCAGCCCGCA GGCCACATTC CTTTCTTCCT GCAG 3'
PstI

EXAMPLE 8

In this example, the sizes of the VNTR alleles at the human chromosome 20 locus D20S15 which are homologous to the human DNA in probe CRI-L355 are determined in five randomly selected individuals by the PCR technique.

DNA was prepared from peripheral blood cells in blood taken from five randomly selected individuals according to standard methods known in the art (see Bell et al., 1981, Proc. Nat'l. Acad. Sci. USA 78: 5759-5763; or Newton et al., 1988, Nucl. Acid. Res. 16: 8233-8243). Synthetic oligonucleotide primers of the following sequence were designed to amplify the VNTR region of the locus homologous to probe CRI-L355 by PCR, and they were synthesized by Operon Technologies (San Pablo, CA) for applicants:

Primer #1: 5' ACAATCTATACTACTAGA 3'

Primer #2: 5' CTCTCTTATTAAACAAGA 3'

Primers #1 and #2 are underlined in the sequence depicted in Example 4.

In order to amplify the VNTR region of the D20S15 locus by PCR, the following items were mixed in a small sterile tube: (1) 8 ul of "5x salts" (83 mM ammonium sulfate, 335 mM Tris-HCl (pH 8.8), 33.5 mM $MgCl_2$), (2) 4 ul of a solution which is 15 mM for each deoxynucleoside triphosphate (dATP, dCTP, dGTP, & dTTP), (3) 4 ul of a solution which is 10 uM in primer #1, (4) 4 ul of a solution which is 10 uM

in primer #2, (5) 0.5 ul of a Taq (Thermus aquaticus) DNA polymerase ("AmpliTaq", Cat. No. N801-0060, Perkin Elmer Cetus, Norwalk, CT) solution consisting of 5 units/ul, (6) 20 ul of sterile deionized water, and (7) 1 ul of whole human DNA at 0.25 ug/ul. The tube was placed in a thermal cycler device such as the Perkin Elmer Cetus DNA thermal cycler (Perkin Elmer Cetus, Norwalk, CT) and subjected to 30 cycles of 91^{o}C for 1.5 min, 43^{o}C for 1.5 min, and 72^{o}C for 3.0 min essentially according to the PCR method described in US patents 4,683,195 and 4,683,202.

Amplification of the samples was stopped by chilling on ice. About 4 ul of each sample was applied to a 2% agarose gel in Tris-acetate-EDTA buffer (Maniatis, Fritsch, & Sambrook, "Molecular Cloning -- a Laboratory Manual", Cold Spring Harbor Press, Cold Spring Harbor, N.Y., pp. 150-162) and subjected to electrophoresis at about 100 volts for about four hours. The DNA was visualized by staining the gel with ethidium bromide (0.5 ug/ml) and subjecting it to illumination with uv light according to standard methods known in the art. As shown in Fig. 1, the DNA from each of the five individuals contains one or two amplified products, and these amplified products differ in migration position in the gel. This is due to the fact that each individual carries a different number of sequence repeats at the D20S15 locus, and each individual carries two such loci, one on each of his chromosome 20 copies.

Both alleles in a single individual are not always visible, perhaps because they migrate at the same or nearly the same position in the gel or because one allele contains so many repeated units as to yield an inordinately large and poorly amplified product. Nevertheless, many of the individuals can be distinguished one from another due to the pattern of their alleles at the D20S15 locus. Clearly, caution must be exercised in interpreting results in which both alleles are not visible. Conclusions cannot be based on the apparent absence of that band. Of course, different electrophoresis conditions, including changes in the % agarose, use of a polyacrylamide gel, length of time of electrophoresis, or the type of buffer, may be used to reveal the second band in many cases.

Incorporation-By Reference

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Equivalents

Those skilled in the art will know or be able to ascertain using no more than routine experimentation many equivalents to the embodiments

of the invention described herein. For example, one of ordinary skill in the art may extend the sequences provided herein for up to 100 bp on either side and use this information to prepare probes or primers. These and all other equivalents are intended to be encompassed by the following claims.

What We claim is:

CLAIMS

1. An oligonucleotide probe being homologous or complementary to only a portion of any of the sequences of Examples 1-7.

2. An oligonucleotide probe as claimed in claim 1, wherein the probe is homologous or complementary only to at least a portion of any of the VNTR regions of Examples 1-7.

3. An oligonucleotide probe as claimed in claim 2, wherein the probe is of a size corresponding to only a single repetitive sequence of the VNTR region.

4. An oligonucleotide probe as claimed in claim 1, wherein the probe is of a size corresponding to at least two contiguous repetitive sequences.

5. An oligonucleotide probe as claimed in claim 4, wherein the probe is of a size corresponding to five or less repetitive sequences.

6. An oligonucleotide probe as claimed in claim 1, wherein the probe is homologous or complementary only to any of the flanking regions of Examples 1-7.

7. An oligonucleotide probe as claimed in claim 1, wherein the probe is homologous or complementary to both a flanking region and a VNTR region contiguous with one another.

8. A oligonucelotide probe as claimed in claim 1 having a sequence corresponding to at least a portion of the sequence depicted in Example 1.

9. An oligonucleotide as claimed in claim 1 having a sequence corresponding to at least a portion of the sequence depicted in Example 2.

10. An oligonucleotide as claimed in claim 1 having a sequence corresponding to at least a portion of the sequence depicted in Example 3.

11. An oligonucleotide as claimed in claim 1 having a sequence corresponding to at least a portion of the sequence depicted in Example 4.

12. An oligonucleotide as claimed in claim 1 having a sequence corresponding to at least a portion of the sequence depicted in Example 5.

13. An oligonucleotide as claimed in claim 1 having a sequence corresponding to at least a portion of the sequence depicted in Example 6.

14. An oligonucleotide as claimed in claim 1 having a sequence corresponding to at least a portion of the sequence depicted in Example 7.

15. An oligonucleotide probe capable of selectively hybridizing to only a VNTR region of a locus selected from the group consisting of D18S17, D1S47, D20S15, D21S112, D6S22, and D11S129.

16. An oligonucleotide probe capable of selectively hybridizing to a VNTR region, a flanking region or both and being of a size of less than 100 base pairs.

17. A primer for initiating the synthesis of an extension product along a strand of DNA and amplifying a VNTR region comprising an oligonucleotide capable of hybridizing to the flanking region of a VNTR region of a locus selected from the group consisting of D18S17, D1S47, D20S15, D21S112, D6S22, and D11S129, and wherein the primer is not capable of hybridizing to only the VNTR region under PCR extension conditions.

18. A primer for initiating the synthesis of an extension product along a strand of DNA comprising an oligonucleotide capable of hybridizing to the flanking region of a VNTR region of a locus selected from the group consisting of D18S17, D1S47, D20S15, D21S112, D6S22, and D11S129, and wherein the

primer has a sequence substantially non-complementary to and non-homologous with the nucleotide sequences of the VNTR regions set forth in Examples 1-7.

19. A primer for initiating the synthesis of an extension product along a strand of DNA as claimed in either of claims 15 or 16 wherein the oligonucleotide is complementary to or is homologous with at least a portion of any one of the flanking sequences of Examples 1-7.

20. A primer for initiating the synthesis of an extension product as claimed in claim 19 wherein the oligonucleotide is complementary to or is homologous with a portion of the flanking sequences of Examples 1-7 within 100 base pairs of the VNTR regions of Examples 1-7.

21. A primer for initiating the synthesis of an extension product as claimed in claim 19 wherein the primer has a length of between 15 and 30 nucleotides.

22. A pair of oligonucleotide primers adapted to interact with a common segment of DNA to amplify a region of a locus, comprising

a first oligonucleotide primer having a sequence complementary with at least a portion of the flanking region at one end of a VNTR region of a first strand of the locus, and

a second oligonucleotide primer having a sequence complementary with at least a portion of the flanking region at the opposite end of the VNTR region of a second strand of the locus, the second strand being complementary to the first strand, wherein said first and second primers are non-homologous and non-complementary and wherein said locus is selected from the group consisting of D18S17, D1S47, D20S15, D21S112, D6S22, and D11S129.

23. A pair of oligonucleotide primers as claimed in claim 22 wherein said primers are not capable of hybridizing to only VNTR regions under PCR extension conditions.

24. A pair of oligonucleotide primers as claimed in claim 22 wherein said primers having nucleotide sequence substantially non-homologous and non-complementary to the nucleotide sequences of the VNTR regions set forth in Examples 1-7.

25. A pair of oligonucleotide primers as claimed in claim 24 wherein said primers define the region of the locus amplified, and wherein the unique single copy portion of the amplified portion represents less than 50 percent of the size of the entire amplified portion.

26. A pair of oligonucleotide primers as claimed in claim 24 wherein each of the primers has a length between 15 and 30 nucleotides.

27. A pair of oligonucleotide primers as claimed in claim 24 wherein the unique single copy portion of the amplified portion is of a size which does not interfere with the detection of the number of repeat sequences in the amplified portion.

28. A pair of oligonucleotide primers as claimed in claim 24 wherein said primers define an amplified region of a locus and wherein said primers are homologous or complementary to a portion of the flanking region within 100 base pairs of the VNTR regions.

29. A pair of oligonucleotide primers as claimed in claim 24 wherein said primers define an amplified region of a locus and wherein said primers are homologous or complementary to a portion of the flanking region within 60 base pairs of the VNTR regions.

30. A pair of oligonucleotide primers as claimed in claim 22 wherein the first and second primers have nucleotide sequences homologous or complementary to at least a portion of the flanking sequence of Example 1.

31. A pair of oligonucleotide primers as claimed in claim 22 wherein the first and second primers have nucleotide sequences homologous to at least a portion of the flanking sequence of Example 2.

32. A pair of oligonucleotide primers as claimed in claim 22 wherein the first and second primers have nucleotide sequences homologous or complementary to at least a portion of the flanking sequence of Example 3.

33. A pair of oligonucleotide primers as claimed in claim 22 wherein the first and second primers have nucleotide sequences homologous or complementary to at least a portion of the flanking sequence of Example 4.

34. A pair of oligonucleotide primers as claimed in claim 22 wherein the first and second primers have nucleotide sequences homologous or complementary to at least a portion of the flanking sequence of Example 5.

35. A pair of oligonucleotide primers as claimed in claim 22 wherein the first and second primers have nucleotide sequences homologous or complementary to at least a portion of the flanking sequence of Example 6 .

36. A pair of oligonucleotide primers as claimed in claim 22 wherein the first and second primers have nucleotide sequences homologous or complementary to at least a portion of the flanking sequence of Example 7.

37. A method for determining the genetic identity between two samples of DNA comprising,

treating a first sample of DNA to produce fragments of DNA or amplified products thereof, said fragments having at least one VNTR region of a locus selected from the group consisting of D18S17, D1S47, D20S15, D21S112, D6S22, and D11S129 present in its entirety as a contiguous piece of DNA;

separating the DNA fragments of the sample based upon the size of the VNTR regions and producing a fingerprint of said first sample;

repeating the above steps with a second sample of DNA; and

comparing the fingerprint of the first sample of DNA to that of the second sample of DNA to establish or exclude identity of the two DNA samples.

38. A method for determining the genetic identity between two samples of DNA comprising:

a) digesting a first sample DNA with enzymes thereby producing fragments of DNA, said enzymes are selected to cut the DNA samples such that at least one VNTR of locus selected from a group consisting of D18S17, D1S47, D20S15, D21S112, D6S22, and D11S129 is present in its entirety as contiguous piece of DNA;

b) placing the digested fragments of DNA on a gel under conditions which allow the fragments of DNA to move to positions within the gel based upon their size thereby creating a pattern of bands;

c) contacting the pattern of bands with at least one labelled probe capable of selectively hybridizing to only a VNTR region of the locus selected from the group consisting of D18S17, D1S47, D20S15, D21S112, D6S22, and D11S129;

d) repeating steps (a) through (c) for a second sample of DNA; and

e) comparing the sizes of the VNTR regions from both samples to establish or exclude identity of the DNA.

39. A method for determining the genetic identity between two samples of DNA comprising:

a) digesting a first sample DNA with enzymes thereby producing fragments of DNA, said enzymes are selected to cut the DNA samples such that at least one VNTR of locus selected from a group consisting of D18S17, D1S47, D20S15, D21S112, D6S22, and D11S129 is present in its entirety as contiguous piece of DNA;

b) placing the digested fragments of DNA on a gel under conditions which allow the fragments of DNA to move to positions within the gel based upon their size thereby creating a pattern of bands;

c) contacting the pattern of bands with at least one labelled probe capable of hybridizing to a nucleic acid sequence complementary or homologous to only a portion of any of the sequences of Examples 1-7;

e) repeating steps (a) through (d) for a second sample of DNA; and

f) comparing the sizes of the VNTR regions from both samples to determine the existence or nonexistence of identity of the DNA.

40. A method as claimed in claim 49 further comprising the transfer of the DNA from the gel to a substrate while maintaining the pattern of bands prior to contacting the pattern of bands with at least one labelled probe.

41. A method as claimed in claim 39 wherein the substrate is contacted with an oligonucleotide probe having a sequence corresponding to at least a portion of the sequences depicted in Example 1.

42. A method as claimed in claim 39 wherein the substrate is contacted with an oligonucleotide probe having a sequence corresponding to at least a portion of the sequences depicted in Example 2.

43. A method as claimed in claim 39 wherein the substrate is contacted with an oligonucleotide probe having a sequence corresponding to at least a portion of the sequences depicted in Example 3.

44. A method as claimed in claim 39 wherein the substrate is contacted with an oligonucleotide probe having a sequence corresponding to at least a portion of the sequences depicted in Example 4.

45. A method as claimed in claim 39 wherein the substrate is contacted with a oligonucleotide probe having a sequence corresponding to at least a portion of the sequences depicted in Example 5.

46. A method as claimed in claim 39 wherein the substrate is contacted with an oligonucleotide probe having a sequence corresponding to at least a portion of the sequences depicted in Example 6.

47. A method as claimed in claim 39 wherein the substrate is contacted with an oligonucleotide probe having a sequence corresponding to at least a portion of the sequences depicted in Example 7.

48. A method as claimed in claim 39 wherein the substrate is contacted with an oligonucleotide probe which selectively hybridizes only to a VNTR region.

49. A method as claimed in claim 39 wherein the substrate is contacted with an oligonucleotide probe of a size corresponding to a single repetitive sequence.

50. A method as claimed in claim 39 wherein the subtrate is contacted with an oligonucleotide probe of a size corresponding to at least two of contiguous repetitive sequences.

51. A method as claimed in claim 39 wherein the substrate is contacted with an oligonucleotide probe which selectively hybridizes only to both a flanking region and a VNTR region contiguous with one another.

52. A method as claimed in claim 39 wherein the substrate is contacted with oligonucleotide probes for at least two different loci

53. A method for determining identity comprising,

amplifying the VNTR region of a first sample of DNA, the VNTR region being within a locus selected from the group consisting of D18S17, D1S47, D20S15, D21S112, D6S22, and D11S129,

separating the amplified DNA from other DNA in the sample based at least in part upon the size of the amplified DNA,

amplifying the corresponding VNTR region of a second sample DNA,

separating the amplified DNA of the second sample based at least in part of the size of the amplified DNA of the second sample, and

comparing the size of the VNTR regions of the first and second samples of DNA.

54. A method as claimed in claim 53 wherein the VNTR regions are amplified by PCR using primers to the flanking regions of said VNTR regions.

55. A method as claimed in claim 54 wherein the amplified DNA are separated from the other DNA in gels, and wherein the sizes of the VNTR regions are determined by staining the DNA within the gels and locating the position of the amplified DNA with the gels.

56. A method as claimed in claim 54 wherein the VNTR region amplified corresponds to a sequence selected from the group consisting of the VNTR sequences of Examples 1-7.

57. A method as claimed in claim 55 wherein the VNTR regions of at least two different loci are amplified.

58. A method as claimed in claim 54 wherein the VNTR regions are amplified using primers corresponding to at least a portion of the flanking sequences of Examples 1-7.

59. A method as claimed in claim 58 wherein primers having a length of between 15 and 30 base pairs are used.

60. A method as claimed in claim 54 wherein the VNTR regions are amplified using primers corresponding to at least a portion of the flanking sequences of Examples 1-7 and wherein said portion of the flanking sequences is within 100 base pairs of VNTR region associated therewith.

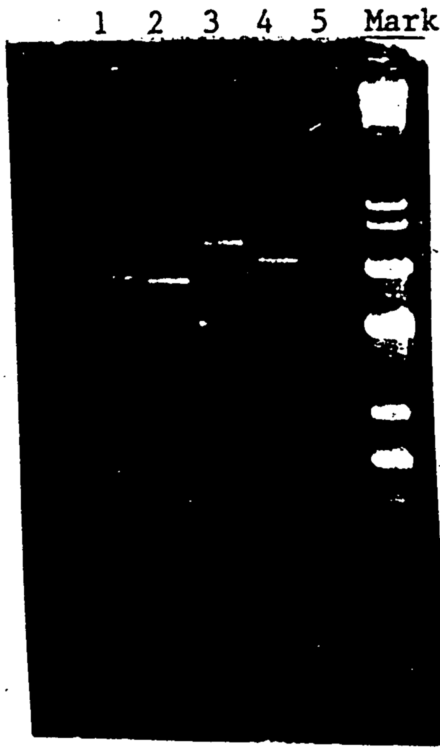

FIG. 1

INTERNATIONAL SEARCH REPORT

International Application No PCT/US91/00196

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): C12Q 1/70; C07H 21/04; G01N 33/53
U.S. CL.: 536/27; 435/6; 935/77,78

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
U.S. CL.	435/6; 935/77,88; 536/27

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

1) Sequence Search In Genbank and Uembl
2) Online Search In Biosis
3) Online Search In APS (Automated Patent System)

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
T	US,A, 4,987,066 (EPPLEN) 22 JANUARY 1991. See claims 1-26.	1-60
T	US,A, 4,994,368 (GOODMAN et al) 19 FEBRUARY 1991. See columns 3-6.	37-60
P,Y	US,A, 4,965,188 (MULLIS ET AL) 23 OCTOBER 1990. See claims 1-50.	37-60
A	Nucleic Acids Research, Volume 17, No.13, issued 1989, (LONDON, GB), Armour et al., "Sequences Flanking The Repeat Arrays Of Human Minisatellites: Association With Tandem And Dispersed Repeat Elements, pages 4925-4935, see entire document.	1-60
A	Proc. Natl. Acad. Sci., Volume 79, issued June 1982, (Washington, D.C.) Epplen et al. "Base sequence of a cloned Snake W-Chromosome DNA Fragment and Identification of a Male-specific putative mRNA in the mouse,	1-60

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

23 APRIL 1991

Date of Mailing of this International Search Report

24 MAY 1991

International Searching Authority

ISA/US

Signature of Authorized Officer

For GARY L. KUNZ

Nguyen Ngoc-Ho

NGUYEN NGOC-HO
INTERNATIONAL DIVISION

Form PCT/ISA/210 (second sheet) (Rev.11-87)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	pages 3798-3802, see entire document.	1-60
A	Nucleic Acids Research, Volume 16, No.17, issued 1988, Ali et al "Intrisic polymorphism of variable number tandem repeat loci in the human genome." See pages 8487-8496. see entire document.	1-60
A	EMBO Journal, Volume 5, No.8, issued 1986, JARMAN et al "Molecular Characterisation of a Hypervariable Region Downstream of the Human -globin gene cluster," pages 1857-1863. see entire document.	1-60
A	American Journal of Human Genetics, Vol. 32, issued 1980, BOTSTEIN et al. "Construction of a Genetic Linkage Map in Man Using Restriction Fragment Length Polymorphisms." pages 314-331. see entire document.	1-60
A	Proc. Natl. Acad. Sci., Volume 77, No.11, issued November 1980, Wyman et al. "A Highly polymorphic locus in Human DNA." pages 6754-6758. see entire document.	1-60